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IN THE SPECIFICATION:

[0037] Experimental tests ~~were~~ are conducted using H. zea caterpillars on leaves of tobacco plants (Nicotiana tabacum). The leaves ~~were~~ are fully expanded and equal in size. Each caterpillar possesses spinnerets which are the principal secretory structures of the labial salivary glands. The H. zea caterpillars ~~were~~ are divided into two groups. In one group, the spinnerets ~~were~~ are destroyed to prevent secretion of saliva. In the other group, the spinnerets ~~were~~ are left intact. The caterpillars of both groups ~~were~~ are each placed on a fully expanded leaf of a tobacco plant, respectively, and allowed to feed for about 3 days. The caterpillars ~~were~~ are then removed and the leaves ~~were~~ are individually ground. The ground leaves ~~were~~ are then analyzed by liquid chromatography using aqueous extraction thereof with the alkaloids separated on a reverse phase column. Results of the analysis indicate a median nicotine reduction of about 26% in tobacco leaves fed by intact caterpillars as compared to the leaves fed by the caterpillars with destroyed spinnerets.

[0038] Four groups of Individual tobacco leaves ~~were~~ are each treated with one of four test solutions containing glucose oxidase, raw salivary gland extract of H. zea, heat treated (inactive) glucose oxidase, or a water control. The leaves receiving the salivary gland extract ~~were~~ are administered about 20 ng of glucose oxidase. The leaves were incubated for about 3 days. The results are shown in Table 1 below.

[0039] As indicated in Table 1, leaves treated with glucose oxidase and salivary extract each ~~exhibited~~ exhibit significant reductions in nicotine over the control and the heat treated glucose oxidase in which glucose oxidase is rendered substantially inactive due to the application of heat. The leaves treated with active GOX ~~showed~~ show a nicotine reduction of about 0.60-0.70 mg/g, while the leaves treated with the salivary extract ~~showed~~ show a nicotine reduction of about 0.70-0.80 mg/g.

[0040] Using the process and data obtained from Examples 1 and 2, mature tobacco plants (N. tabacum) ~~were~~ are cultivated on a quarter acre plot. One group of the tobacco plants ~~was~~ is exposed to H. zea neonates for a three day period during the growing season. A second group of the tobacco plants ~~was~~ is exposed to H. zea neonates multiple times each for a three-day period during the growing season. A third group of tobacco plants ~~was~~ is isolated from H. zea neonates for establishing a control. The leaves ~~were~~ are harvested at the end of the growing season and the caterpillars ~~were~~ are removed. The tobacco leaves ~~were~~ are air dried and processed. Each of the dried tobacco leaves ~~were~~ are treated and extracted with 10 ml of 25 mM sodium phosphate buffer at 30°C for about 24 hours at constant agitation. The extract ~~was~~ is then filtered and diluted prior to passage into a high performance liquid chromatograph using procedures outlined in Saunders et al. (1981) J. Chromatogr. 205, 147-154, the content of which is incorporated herein by reference. The results of the elution profile ~~showed~~ show that the first group ~~exhibited~~ exhibit reduced foliar nicotine levels of over 26% as compared to undamaged leaves of the control group. The second group of tobacco plants

exposed to multiple treatments ~~exhibited~~ exhibit significantly greater reduction in foliar nicotine levels of from about 50% to 75% as compared to the undamaged leaves of the control group.

[0041] In a manner similar to Example 3, a half acre plot of suitable tobacco growing soil ~~was~~ is divided into two plots [A and B]. Mature tobacco plants ~~were~~ was cultivated as in Example 3 in one quarter acre plot (A) and ~~yielded~~ yield foliar nicotine levels of 0.15-0.075 mg/gram of tobacco for use in cigarettes. The latter nicotine levels are equivalent to using the tobacco filler in Vector brand cigarettes Quest 1 (Low Nicotine) and Quest 2 (Extra low Nicotine) each of which ~~has been~~ is subjected to two (2) "caterpillar treatments".

[0042] In the other quarter acre plot (Plot B), tobacco leaves are grown by the process described in U.S. Patent 6,008,436. The means for transforming plant tissue to yield low nicotine content tobacco plants can be performed by DNA mediated transformation by a ~~bacterial~~ bacteria containing Ti plasmid which transforms the susceptible plant cell capable of regeneration into the required plant. Another approach in producing a transgenic plant is to use microparticles for ballistic transformation to produce the transgenic tobacco plant.

[0043] The tobacco leaves produced in the transgenic plant ~~were~~ are subjected to analysis as in Example 3 with a Quest "Nicotine Free" nicotine content of 0.05 mg per gram reported. With one 75% caterpillar H. zea reduction treatment or a GOX –

leaf bruising treatment, the nicotine content ~~was~~ is reduced to 0.01 mg of nicotine/per gram, the threshold for avoiding addiction by smoking. Depending on the efficiency of the transgenic operation and the nicotine content of the resultant dried tobacco two or more treatments may be required to attain the threshold nicotine requirement.

[0045] Twenty test subjects ~~each~~ are were divided into two groups and asked to smoke two packs of cigarettes per day each of A (0.15) and A (0.075) for a period of two weeks. Group A (0.15) ~~had~~ has a group of 8 of 10 who ~~indicated~~ indicate a desire to continue smoking when offered an opportunity to do so. Group A (0.075) ~~had~~ has 6 of 10 individuals who ~~desired~~ desire to continue smoking.

[0046] Ten test subjects ~~were~~ are asked to smoke two packs per day each of Quest 3 "Nicotine Free" cigarettes for two weeks. The tobacco in 20 cartons of Quest 3 ~~was~~ is treated with a 75% "GOX" treatment and dried and reassembled into 20 cartons. Additionally, 20 cartons of transgenic tobacco ~~was~~ is treated with a 75% "H. zea" approach and ten other test subjects ~~were~~ are asked to smoke two packs per day for two weeks. The "Quest 3" group of ten ~~had~~ has one individual who ~~was~~ is reluctant to stop smoking. The "H. zea" test subjects ~~had~~ has two individuals who ~~have continued~~ continue ~~smoking~~ to smoke.

[0047] Glucose oxidase (GOX) extracted from Aspergillus niger ~~was~~ is obtained from a commercial source Calzyme Laboratories, Inc. B443 Miguelito Court, San Luis

Obispo, California 93401. The molecular weight of GOX ~~was~~ is measured to be about 160,000 comprising a flavin containing a glycoprotein. Solutions containing GOX and water ~~were~~ are prepared in a ratio of 10 µl of water to 20 ng of GOX (90-95%). The GOX activity ~~was~~ is measured at about 200 to 250 U/mg for GOX derived from A. niger in dry powder form. The value U is the amount of enzyme required to oxidize one micromole of glucose per minute at about 25°C and pH=7.

[0048] Forty gallons of the solution based on the above ratio ~~were~~ are prepared in a 55 gallon stainless steel drum. A spray device comparable to commercially available garden sprayers or oscillators ~~were~~ are used to apply the solution on a quarter acre of genetically modified N. tabacum plants as described in U.S. Pat. No. 6,423,520. One day prior to the spray application, the leaves ~~were~~ are slightly damaged with cutting tools. The leaves ~~were~~ are harvested at the end of three to five days. The tobacco leaves ~~were~~ are treated with a 75% "GOX" treatment and air dried and processed into cigarettes containing no fillers.

[0049] The cigarettes ~~were~~ are smoked by 10 test subjects with restrictions similar to Example 5. In these tests only one subject ~~expressed~~ expresses a desire to continue smoking.

[0050] We have discovered that generic defense mechanisms are elicited by herbivores such as caterpillars. In this example, a caterpillar (Pieris brassicae) ~~attacked~~ attacks a cabbage plant releasing a defensive mixture of volatiles which

attract parasitic wasps (Cotesia glomerate) which then attack and destroy the caterpillars. The caterpillar gut regurgitant contains enzymatic β-glucosidase which elicits the mixture of volatiles referred to above. Commercial β-glucosidase performs in a similar manner.

[0051] Cabbage (eight weeks old) and P. Brassicae (caterpillar) and parasitoids (wasps) ~~were~~ are reared according to the method of Steinberg S. et al., Entomol. Exp. Appl 63 163-175 (1992). In the experiments the amount of β-glucosidase in 25 µl clearly ~~resulted~~ results in the attraction of parasitoids (wasps). Ion chromatograms ~~identified~~ identifies (E)-2 hexanol, 1-hexanol, E-2-hexene 1-YL acetate as major components of the volatiles released by the cabbage plants. This experiment illustrates another specific example (compare to H. zea) of evolutionary arms race wherein an elicitor-antagonist biological system focuses on a defensive enzyme reaction.